THE TURNOVER OF THE PROTEIN COMPONENTS OF THE INNER AND OUTER MEMBRANE FRACTIONS OF RAT LIVER MITOCHONDRIA

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The mechanism of mitochondrial turnover and its relationship to the process of mitochondrial biogenesis is still uncertain. Fletcher and Sanadi (1961) reported that several protein components and lipids of rat liver mitochondria had an identical turnover rate. This suggested to these workers that liver mitochondria were labile and were broken down as an entity. Subsequent studies of Beattie, Basford, and Koritz (1967a) indicated that several mitochondrial protein subfractions had an essentially similar half-life, although the soluble proteins of kidney mitochondria had a more rapid turnover. In a recent study, Gross, Getz and Rabinowitz (1969), have also reported that rat liver mitochondrial DNA has a half-life of 9.4 days which is similar to that for the proteins previously published.

The inner and outer mitochondrial membranes appear to have different routes of biosynthesis. Isolated mitochondria are capable of incorporating amino acids only into certain insoluble proteins of the inner membrane (Beattie, Basford and Koritz, 1967b). In kinetic studies on the biosynthesis of rat liver mitochondria, *in vivo*, Beattie (1969) reported that the proteins and lecithin of

the outer membrane were labeled more rapidly than those of the inner membrane suggesting that the outer membrane may be the first mitochondrial component synthesized during mitochondrial biogenesis. This, in turn, suggested the possibility that the inner and outer membranes might have different turnover rates. In the present study the turnover rate of the inner and outer membrane fractions were compared. The proteins of the outer membrane had a half-life of 7.0 days compared to one of 8.4 days for the intact inner membrane-matrix fraction, while the KCl-insoluble proteins of the inner membrane had a half-life of 10.2 days. It is of some interest that this fraction contains the proteins which are synthesized by the mitochondria *in vitro*.

EXPERIMENTAL METHODS

Twenty microcuries of uniformly labeled (^{14}C) L-leucine (250mc/mmole) were injected intravenously into male rats weighing 150-200 gms and the animals killed at 1,3,7,10, and 14 days after injection. Liver mitochondria were prepared and fractionated into inner and outer membrane fractions as previously described (Beattie, 1969).

A representative fractionation of rat liver mitochondria into inner membrane-matrix, outer membrane and soluble fractions by the method of Schnaitman and Greenawalt (1968) is shown in Table I. Approximately 65% of the mitochondrial protein was associated with the inner membrane-matrix fraction as well as 78% of the succinic dehydrogenase and 84% of the isocitric dehydrogenase. Between 10 and 15% of rotenone insensitive DPNH-cytochrome *c* reductase, a specific marker for the outer membrane, was present in the inner membrane-matrix fraction, while the bulk of the activity was concentrated in the outer membrane and soluble fractions. The soluble fraction also contained 15% of the matrix as indicated by isocitric dehydrogenase, while the outer membrane fraction contained 10% of

TABLE I

DISTRIBUTION OF PROTEIN AND ENZYMIC ACTIVITIES AFTER A FRACTIONATION OF RAT LIVER MITOCHONDRIA BY DIGITONIN

	% Total Protein	Isocitric Dehydrogenase mμ moles/min/mg	% Total	Succinic Dehydrogenase mμ moles/min/mg	% Total	DPNH Cytochorome c Reductase mμ moles/min/mg	%Total
Mitochondria	100	62.2	100	38.4	100	138	100
Inner Membrane-Matrix	64.6	81.9	84	56.0	78	27	10
Outer Membrane	9.7	-	-	7.8	10	480	35
Soluble	25.4	59.7	15	-	-	360	55
Inner Membrane-Subfractions							
Matrix	26.4	-	68	-	-	-	-
Residue	63.6	-	-	-	79	-	-

the succinic dehydrogenase, a specific inner membrane marker. The inner membrane-matrix was further fractionated by extraction with water and 0.6 N KCl (Beattie et al., 1966). After these extractions the mitochondria were sedimented at 27,000 x g for 15 minutes. Almost 70% of the isocitric dehydrogenase activity, a marker enzyme for the matrix, was released into the supernatant fraction by this procedure, while 80% of the tightly-bound membrane protein succinic dehydrogenase was sedimented.

RESULTS AND DISCUSSION

The turnover data for the mitochondria and the various submitochondrial fractions is compared in Table II. The proteins of the outer membrane and the soluble fraction had a half-life of 7.0 and 6.8 days respectively. The intact inner membrane-matrix fraction had a half-life of 8.4 days; however, the difference between this and that of the other fractions is of doubtful significance (p=0.2).

TABLE II

TURNOVER OF PROTEIN COMPONENTS OF RAT LIVER MITOCHONDRIA

Regression coefficients and their 95% confidence limits were calculated as described by Goldstein (1964).

Fraction corresponding to	Regression Coefficient per day	95% Confidence Interval	$T_{\frac{1}{2}}$-days
Mitochondria	-0.0373	±0.0132	8.00
Inner Membrane-Matrix	-0.0357	±0.0119	8.40
Outer Membrane	-0.0426	±0.0086	7.00
Soluble	-0.0444	±0.0132	6.78
Inner Membrane Subfractions			
Water-Soluble	-0.0367	±0.0098	8.20
KCl-Soluble	-0.0433	±0.0139	6.95
KCl-Insoluble	-0.0293	±0.0091	10.2
Microsomes	-0.0708	±0.0146	4.25

Most of the remaining submitochondrial fractions have half-lives which do not differ significantly. Of some interest, however, is the 10.2 day half-life of the KCl-insoluble proteins of the inner membrane. This differs significantly ($p=0.02$) from that of the outer membrane or soluble fractions. Figure 1 compares the regressior line of the KCl-insoluble proteins of the inner membrane and the outer membrane. It should be noted that the KCl-insoluble fraction

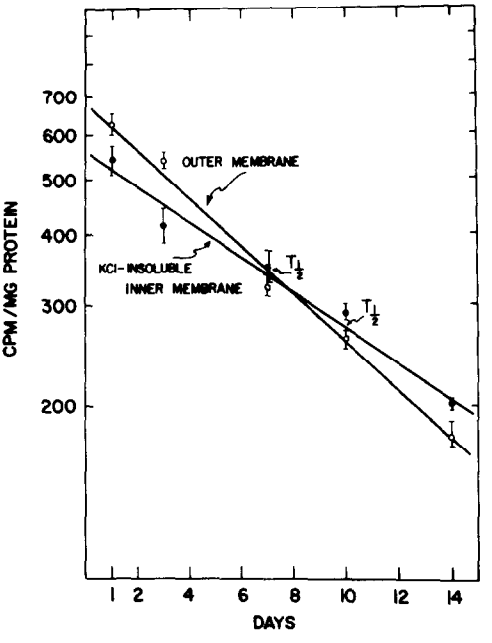

Figure 1. Decline in radioactivity of outer membrane proteins o-o and KCl-insoluble proteins of the inner membrane o-o. Each point is the average of 4 animals. The regression lines were drawn by the method of least squares.

contains proteins which appear to be synthesized by the mitochondria themselves as evidence by the incorporation of amino acids into this protein fraction in isolated mitochondria (Beattie et al., 1967b) and by studies of the labeling of these proteins in rat liver slices (Beattie, 1968). The longer half-life of these proteins may thus result from the presence of an intramitochondrial pool of radioactive amino acid which is reutilized during the time studied.

Alternatively, this fraction may contain a "primary" membrane (Omura, Siekivitz, and Palade, 1967) which is more long-lived than other mitochondrial proteins.

The fact that a difference in turnover of rat liver mitochondrial protein components was not observed in previous studies (Beattie et al., 1967a; Fletcher and Sanadi, 1961; Swick, Rexroth and Strange, 1968) may have resulted from the cruder separations employed in which a protein with a different half-life was only a small proportion of a fraction and hence its turnover masked. In kidney and brain mitochondria, however, a significantly faster turnover of soluble proteins was observed (Beattie et al., 1967a). In addition, several inducible enzymes located in the mitochondria, have been shown to have much more rapid turnover rates than the majority of the mitochondrial proteins (Swick et al., 1968).

The more rapid turnover of the proteins of the outer membrane may be a reflection of its more rapid in vivo synthesis (Beattie, 1969) or alternatively its close relationship with the endoplasmic reticulum (Parsons et al., 1967). The half-life (7.0 days) of the outer membrane fraction is much greater than that of the microsomes (4.25 days) indicating that the outer membrane is not identical with the endoplasmic reticulum. Druyan, De Bernard, and Rabinowitz (1969) have recently indicated that the heme of cytochrome b_5, localized exclusively in the outer membrane, has a significantly shorter half-life than the hemes of cytochrome b and c, inner membrane hemeproteins. It would thus appear that many mitochondrial proteins, perhaps the majority of the inner membrane proteins, do turn over as an entity, but that the outer membrane plus other proteins such as the inducible enzymes may be inserted and deleted from the mitochondrial structure independently of the bulk of the proteins.

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